Cyclosporine Monitoring and Pharmacokinetics in Pediatric Liver Transplant Patients

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THE DEVELOPMENT of cyclosporine (CyA) as a novel immunosuppressive agent has dramatically advanced orthotopic liver transplantation (OLT). The use of CyA in pediatric OLT patients has been hindered by a lack of information concerning the drug's pharmacokinetics in children and the interpretation of blood concentrations during therapy. OLT should have a major effect on CyA's absorption, distribution, metabolism, and excretion since CyA is fat-soluble, highly protein-bound, completely metabolized, and excreted in the bile.[1] Presently, two methods are available for measuring CyA in biological fluids. The two drug assays available produce different results[2] and measure different components of CyA activity. The primary objectives of this study were (1) to examine the bioavailability and pharmacokinetics of CyA in children receiving OLTs and (2) to examine the relationship between drug assay results and the biochemical and clinical status of the pediatric OLT patients.

MATERIALS AND METHODS

Patients

All pediatric patients undergoing OLT procedures were considered eligible for the study. Blood concentrations of CyA were monitored by both radioimmunoassay (RIA) and high-pressure liquid chromatography (HPLC) during therapy in 27 children, following OLT procedures. Blood was obtained daily prior to the morning dose of CyA for at least the first three postoperative days, and then twice weekly until the patient was discharged from the hospital. Other information that was collected included the patient's weight, CyA dosage, hematocrit, WBC count, BUN and serum creatinine, direct and total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and gamma glutamyl transferase (GGT).

Bioavailability and Pharmacokinetics

Bioavailability studies were conducted in 11 pediatric patients. Four patients had suspected CyA bioavailability problems because of low blood concentrations, and seven were studied during the immediate postoperative period. Parental consent was obtained in the children who were studied during the postoperative period. For those studies conducted during the immediate postoperative period, the patient received their prescribed intravenous (IV) CyA dosage, infused over two hours by a Harvard infusion pump on the first study day. Heparinized blood samples were obtained prior to the start of the infusion, at the end of the infusion, and at 4, 6, and 8 hours following the start of the infusion. On the second study day, the patient received the same IV CyA infusion, but also received an oral CyA dose, concurrent with the start of the infusion. The oral CyA liquid was diluted with chocolate milk or juice and administered as quickly as the patient could tolerate. The blood sampling on the second day followed the same schedule as that of the first day. The four additional patients with suspected bioavailability problems were initially studied while on oral CyA and were restudied by either adding or substituting an IV CyA dose at the time of an oral dose. The blood sampling protocol was similar to that described for the postoperative studies. The area under the blood concentration v time curve (AUC) was calculated by the trapazoidal rule. Drug clearance was calculated as the IV CyA dose divided by the AUC (IV), and the percentage of bioavailability (F) was calculated as:

$$F = \frac{AUC\ (IV + oral) - AUC\ (IV)}{AUC\ (IV)} \times \frac{dose\ (IV)}{dose\ (oral)} \times 100.$$

CyA Assay

Blood samples were refrigerated at 4 °C until assayed (in less than seven days). HPLC assays for CyA whole blood concentrations were performed using minor modifications of the technique of Sawchuk and Cartier.[3] Only HPLC analysis was performed on samples for the bioavailability and pharmacokinetic studies. The RIA for CyA whole blood concentrations was performed using material provided by Sandoz Ltd (Basel, Switzerland).

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RESULTS

Twenty-seven children had blood concentrations of CyA monitored by HPLC and RIA during their therapy following OLT. No clear relationship could be established between dose and blood concentration of CyA. For example, although all patients were started on IV CyA in the dose of 2 mg/kg every eight hours, the trough blood concentrations on postoperative day 2 ranged from 418 to 2,516 ng/mL by RIA and from 102 to 648 ng/mL by HPLC. Although the ratio of RIA to HPLC blood concentration varied considerably, four consistent observations were noted. (1) An early elevation in the ratio of two assay results was observed in the first two postoperative weeks. The RIA-HPLC ratio at its highest point in the early postoperative period was 2.5:1.0 to 13.6:1.0, with a mean of 5.2:1.0. (2) The second observation was that deterioration of hepatic function secondary to rejection or hepatic artery thrombosis frequently produced a disproportionate rise in the RIA blood concentration, resulting in an elevated RIA-HPLC ratio. Nine of the 27 children demonstrated a twofold or greater rise in the RIA-HPLC blood concentration ratio concurrent with a change in the clinical status which was associated with an increase in serum bilirubin and GGT, ALT, AST, and alkaline phosphatase. A definitive relationship between rejection, biochemical changes, and the RIA-HPLC relationship could not be established, however. (3) A high RIA-HPLC blood concentration ratio is frequently associated with an elevated BUN level. Sixteen of the 27 children had a BUN level greater than 40 mg/dL, which was frequently associated with an RIA-HPLC ratio of greater than 4:1. This association was common during the immediate postoperative period. One child had an elevated RIA-HPLC ratio 24 days postoperatively that was associated with elevated BUN and serum creatinine levels at a time when serum bilirubin and liver enzymes were within normal limits. In this child, a reduction in CyA dosage was followed by a reduction in the BUN level and RIA-HPLC blood concentration ratio. (4) In patients who remain clinically stable, the ratio of RIA-HPLC blood concentrations stabilizes in the 1.5:1.0 to 3.5:1.0 range.

Table 1 summarizes the bioavailability studies performed in 11 children, following the OLT. Patients A through D were clinically suspected to have CyA malabsorption. Patient A had a large T tube output of bile in the postoperative period, rejection, and a low blood CyA concentration. The CyA clearance was high (13.9 mL/min/kg) despite a total serum bilirubin of 8.2 mg/dL. Patient B had diarrhea and a low blood CyA concentration, and required an oral CyA dose of 75 mg/kg/d to temporarily overcome this problem. Patient C had received an auxiliary OLT, which was not functioning, and had chronic liver failure. Patient D had a low blood CyA level and acute rejection that required retransplanta-

Table 1. Bioavailability in Pediatric OLT Patients

Patient	Age (mo)	Postoperative Time	CyA dose (mg/kg)		Clearance (mL/min/kg)	Bioavailability (%)
			IV	Oral		
A (T tube)	64	4 wk	4.0	13.3	13.9	<5
B (diarrhea)	27	6 wk	1.4	33.3	5.0	6
C (failure)	51	16 wk	1.6	4.7	3.0	5
D (rejection)	47	28 wk	3.1	6.9	4.9	8
1	34	2 d	2.6	8.8	13.0	13
2	67	9 d	1.8	8.9	12.1	18
3	52	3 d	2.4	8.1	7.1	<5
4	39	3 d	1.9	8.1	1.9	<5
5	11	2 d	3.7	18.3	8.2	<5
6	51	3 d	2.0	8.4	10.4	19
7	36	3 d	1.6	9.2	6.0	<5

tion. Patients 1 through 7 were studied during the immediate postoperative period. Patients 1, 3, and 6 were studied following their first OLT procedure, and patients 2, 4, 5, and 7 were studied following their second OLT. The mean CyA blood clearance for the seven patients was 8.4 mL/min/kg. The bioavailability of CyA measured by HPLC was <5% in four of the seven patients and was ultimately associated with retransplantation (two patients), hepatic artery thrombosis, or bowel perforation. The three patients with CyA absorption >5% in the immediate postoperative period also had the highest drug clearance values (>10 mL/min/kg).

DISCUSSION

Preliminary investigations of CyA pharmacokinetics have been conducted in bone marrow[4] and kidney transplant patients.[5] The malabsorption of CyA has been noted previously in kidney transplant candidates and improved over time posttransplantation.[5] While the malabsorption of CyA was expected in the postoperative period following OLT, the high degree of variability was particularly notable. Two patients absorbed nearly 20% of CyA soon following OLT and could therefore be weaned from IV therapy relatively rapidly. The current regimen of early initiation of oral CyA in conjunction with IV CyA while monitoring trough blood concentrations allows a rapid conversion to oral therapy alone in those patients who can absorb the drug.

Larger doses of oral CyA per kilogram of body weight may be more necessary in children than was formerly recognized. The two factors that produce this requirement were an increased CyA clearance in children and frequent malabsorption. Our previous study in adult OLT patients had demonstrated a CyA clearance of 463.0 mL/min or 5.8 mL/min/kg.[6] The observation of a higher mean clearance in children even in the immediate postoperative period is consistent with the higher clearance in children of other highly metabolized drugs such as theophylline. The studies in patients A through D confirm that CyA malabsorption is a problem not only immediately following OLT but during the period of convalescence. While the low CyA blood concentrations in patient A, who had a large bile output from the T tube, suggested a high biliary CyA excretion, subsequent studies have shown that little unmetabolized CyA appears in the bile.[7]

The HPLC assay for CyA measures the parent compound, whereas the RIA detects both parent CyA plus metabolites.[2] Both assays can be used effectively for blood CyA monitoring but some important differences were observed in monitoring children following OLT. Since a large quantity of CyA metabolite detected by RIA is eliminated in bile,[7] periods of poor liver function and decreased bile flow may produce a large discrepancy (a high RIA-HPLC ratio) between the assays. These high RIA-HPLC ratios occurred primarily during the first week following the OLT procedure and during periods of diminished liver function, such as during rejection or following hepatic artery thrombosis. Since the primary metabolites of CyA do not possess significant immunosuppressant activity in animals and have not been tested in humans,[8] the HPLC assay must be used as a monitoring tool in conjunction with the RIA during these periods of diminished liver function. The elevated BUN and serum creatinine levels occasionally noted with a high RIA-HPLC blood CyA concentration ratio suggest the possibility of a nephrotoxic metabolite. While this hypothesis has not been tested, there is a clear advantage to having both assay techniques available when monitoring OLT patients.

SUMMARY

Pediatric patients undergoing OLT have large variations in their dosage requirement of CyA due to variable drug absorption and elimination. CyA malabsorption is common in the period immediately following OLT and in patients with problems such as diarrhea, liver failure, and externalized bile drainage. While

drug clearance is highly variable, it is generally higher in OLT children than in adults, and children may require larger CyA doses on a body weight basis. Monitoring blood concentrations of CyA is essential in OLT patients, and both the RIA and HPLC techniques are useful. However, during the immediate postoperative period and during diminished hepatic function, a high ratio of RIA to HPLC results can be expected. Both assay techniques should therefore be available when monitoring OLT patients.

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